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1 **Chronic feeding exposure to virgin and spiked microplastics**
2 **disrupts essential biological functions in teleost fish**

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25 **Abstract**
26 Toxicity of polyethylene (PE) and polyvinyl chloride (PVC) microplastics (MPs), either
27 virgin or spiked with chemicals, was evaluated in two short-lived fish using a freshwater
28 species, zebrafish, and a marine species, marine medaka. Exposures were performed through
29 diet using environmentally relevant concentrations of MPs over 4 months. No modification of
30 classical biomarkers, lipid peroxidation, genotoxicity or F0 behaviour was observed. A
31 significant decrease in growth was reported after at least two months of exposure. This
32 decrease was similar between species, independent from the type of MPs polymer and the
33 presence or not of spiked chemicals, but was much stronger in females. The reproduction was
34 evaluated and it revealed a significant decrease in the reproductive output for both species and
35 in far more serious numbers in medaka. PVC appeared more reprotoxic than PE as were MPs
36 spiked with PFOS and benzophenone-3 compared to MPs spiked with benzo[a]pyrene.
37 Further, PVC-benzophenone-3 produced behavioural disruption in offspring larvae. These
38 results obtained with two species representing different aquatic environments suggest that
39 microplastics exert toxic effects, slightly different according to polymers and the presence or
40 not of sorbed chemicals, which may lead in all cases to serious ecological disruptions.

41

42

43 **Keywords:** polyethylene, polyvinyl chloride, adsorbed chemicals, growth alteration,
44 reproductive toxicity

45

46 **1. Introduction**

47 Frequently, manufactured plastic debris end up in the oceans and eventually appear as
48 macroplastics (> 5 mm in size), microplastics (MPs; particles between 1 µm and 5 mm in
49 size) or smaller nanoplastics (< 1 µm in size) [1, 2]. MPs consist of either plastic particles
50 designed and produced for specific purposes [3, 4] or small plastic fragments derived from the
51 fragmentation of larger pieces [5-8]. Given the ubiquity of sources and their persistence in the
52 aquatic environment, MPs are globally distributed in various environments including some
53 areas far from emission sources, i.e. Greenland, Antarctica [9-12]. Consequently, they are
54 found ubiquitously in any water body [13-17] and concentrations in marine ecosystems are
55 still increasing [12].

56 The ability to ingest MPs has been documented across a wide range of phyla including e.g.
57 zooplankton, molluscs, marine worms, fish, and cetaceans [18-24]. Ingestion and egestion
58 depends to some extent on MPs size and shape [23, 24]. In fish, the ingestion of MPs was
59 demonstrated at all life stages in field sampling [25-30]. The transfer of MPs through food
60 webs was reported in laboratory studies [31-37].

61 Effects of MPs on marine and freshwater organisms and their potential consequences on
62 ecosystems functioning have been a matter of debate for more than a decade [8, 38-41]. This
63 is related to the fact that the effects of MPs on aquatic organisms are not well understood;
64 particularly, because of the high variety of MPs combined with diverse aging conditions (see
65 [42] for more details). Adverse effects might be attributed to processes, such as the lack of
66 egestion or false satiation after ingestion of MPs devoid of nutritional value [14]. Besides, by
67 interfering with endogenous microbiota, MPs have also been shown to induce a dysbiosis [43-
68 45], which may have consequences on other physiological functions. Some authors reported
69 induction of oxidative stress, inflammatory processes, or physiological stress responses [38,
70 46-56]. However, used exposure conditions (e.g. duration, routes, and concentrations) often
71 raise the question of ecological relevance. At the same time, a growing number of
72 publications, mainly short-term exposure, report that MPs ingestion induced no or limited
73 toxicity [35, 45, 56-61]. Taken all together, these results also suggest that short-term
74 exposures are not relevant to evaluate MPs toxicity and a fortiori to evaluate toxicity at
75 environmental concentrations.

76 Plastic polymers are considered biologically inert. The manufacturing of plastic products uses
77 additives, including phthalates, nonylphenol, bisphenol A and brominated flame retardants, to

78 improve physico-chemical properties. These additives were reported to be transferred to
79 aquatic organisms and to cause toxicity [62-64]. A recent article described a differential
80 toxicity between native particles, “extracted particles”, and additives depending on the type of
81 plastic [65]. In addition to these intrinsic chemicals, it was shown that MP particles sorb
82 significant amounts of organic pollutants from the surrounding environment, such as per- and
83 polyfluoroalkyl substances (PFAS), polycyclic aromatic hydrocarbons (PAHs),
84 polychlorinated biphenyls (PCBs), pesticides, and personal care products. This might be of
85 great concern because these compounds are often toxic and can bioaccumulate [39, 63, 66,
86 67]. While the importance of the role of MPs in the transfer of persistent organic pollutants in
87 biota is still controversial [39, 40, 68], the adsorption of different classes of organic pollutants
88 on the surface of different MP materials was demonstrated under both laboratory and field
89 conditions [52, 69]. In the aquatic environment, organic pollutants are commonly found in the
90 water surface microlayer, where low-density MPs such as polyethylene (PE) are also
91 abundant [55, 67, 70], or in sediments, where high-density MPs such as polyvinyl chloride
92 (PVC) and aged or biofouled low-density MPs accumulate. Perfluorooctane sulfonic acid
93 (PFOS), benzo[a]pyrene (BaP), and benzophenone-3 (BP3) represent different chemical
94 families of organic pollutants frequently detected in aquatic systems. PFOS represents the
95 main perfluorinated alkylated substance that is frequently detected in aquatic environments
96 and is used as a flame retardant in plastic [71]. BaP is a PAH ubiquitously distributed in both
97 coastal and offshore environments. Polycyclic aromatic hydrocarbons are known to adsorb at
98 high rates to different types of MP polymers in seawater [52, 69]. Benzophenone-3 is
99 commonly used as a UV-filter in cosmetics such as sunscreens and is frequently detected in
100 coastal areas. BP3 shows low degradation rates in surface waters [72].

101 In the present study, we used long-term exposure to evaluate the physiological effects of PE
102 and PVC MPs, with and without the presence of PFOS, BaP, or BP3 on two models fish
103 species, a marine one, marine medaka (*Oryzias melastigma*), and a freshwater one, zebrafish
104 (*Danio rerio*). Studied endpoints included biochemical biomarkers, survival, growth,
105 reproduction, and swimming behaviour of exposed fish along with the survival, growth, and
106 swimming behaviour of unexposed F1 offspring. The aim of this experimental design was to
107 compare the toxicity of two plastic polymer types, PE and PVC, and the effects of adsorbed
108 chemicals. An additional aim was to evaluate the genericity of the effects on teleosts as
109 regards essential life-history traits.

110 **2. Materials and methods**

111 **2.1 Husbandry and egg production**

112 Fish brood stocks were routinely maintained in the Laboratoire Ressources Halieutiques,
113 Ifremer (facility authorization A171901; project authorization APAFIS#10883). Brood stocks
114 were reared in isothermal rooms with a 14/10 h light/dark photoperiod in recirculating
115 systems using conditions that guaranteed that ammonia, nitrite, and nitrate levels remain
116 within recommended ranges [73]. Additional details about rearing can be found in
117 Supplementary materials.

118 Experimental groups were built in similar ways with both species: eggs from several group-
119 spawns (medaka) or pair-spawns (zebrafish) with fertilisation rates above 80% were kept. The
120 day after collection, eggs from different spawns (at least 6 spawns per replicate) were evenly
121 distributed in order to avoid potential biases due to individual spawns. Sixty eggs were
122 transferred to the appropriate number of 10 cm Petri dishes and dishes were incubated at 28
123 °C under a 14 h/10 h light/dark cycle. Daily care of the Petri dishes consisted of removing
124 dead eggs and changing approximately 20% of water. After hatching, starting at 8 days post-
125 fertilisation (dpf) for medaka and at 72 h post-fertilization (hpf) for zebrafish, chorions were
126 removed from the Petri dishes. Further rearing was performed as described previously [74].
127 Briefly, hatched larvae were then transferred to 1 L tanks for 1 week and then to 3 L tubes
128 located in independent 10 L tanks in a dedicated flow-through rearing system with a hourly
129 automatic water exchange (total daily exchange: 30% of the volume). Two weeks later, at 1
130 month, tubes were gently poured in their 10 L tanks. On this occasion, fish were distributed at
131 random to produce per treatment three replicate tanks for medaka and six replicate tanks for
132 zebrafish tanks, containing each 30 individuals to reach the density of 3 fish/L.

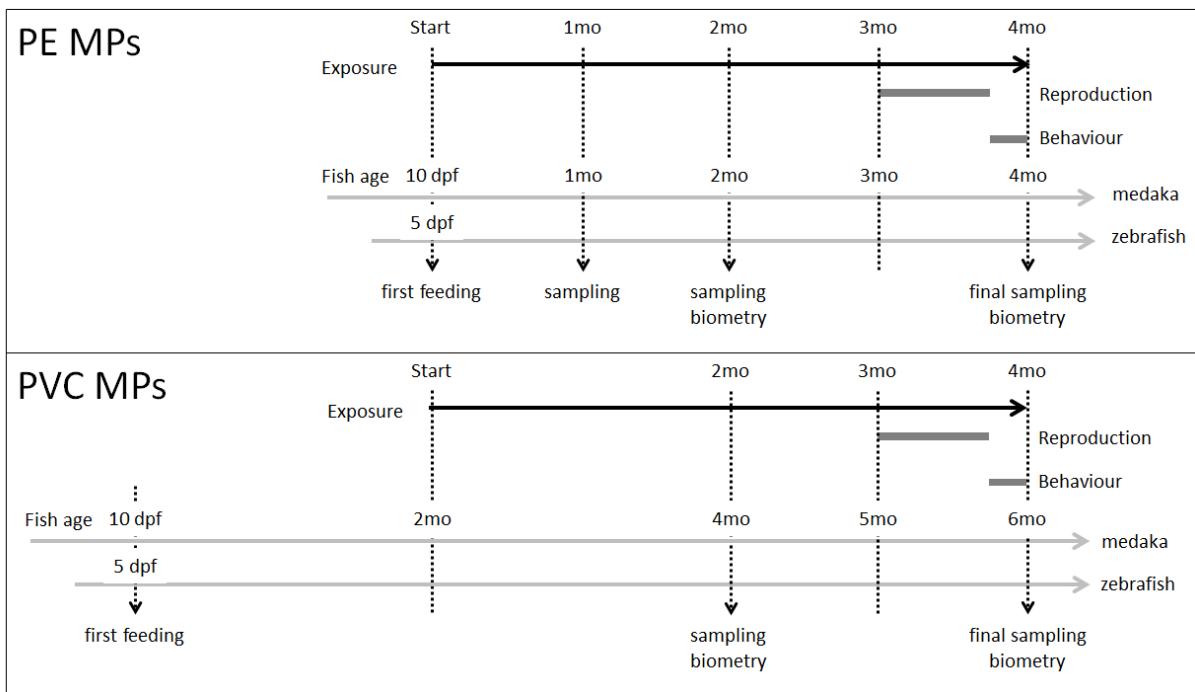
133 **2.2 Microplastics, experimental design and diet preparation**

134 Polyethylene MPs were purchased from Micro Powders Inc. (order no. MPP-635 G; New
135 York, USA) for a given size range of 11-13 µm. PVC MPs were purchased from Fainplast
136 (Ascoli Piceno AP, Italia) for a given size range of 125-250 µm. Polymer of MPs were
137 confirmed by FTIR/UATR. PFOS (1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluorooctane-1-
138 sulfonic acid; CAS 2785-37-3; purity ≥ 98%), BaP (benzo[a]pyrene; CAS 50-32-8; purity
139 ≥ 96%) and BP3 ((2-hydroxy-4-methoxyphenyl)-phenylmethanone; CAS 131-57-7; purity
140 ≥ 98%) were purchased from Sigma Aldrich (Stockholm, SE). Solvent used were deionized
141 water for PFOS and toluene (purity ≥ 96%; Solveco) for BaP and BP3. All other chemicals

142 and reagents were purchased at the highest purity available from Sigma-Aldrich, unless stated
143 otherwise.

144 Polyethylene and PVC were spiked with PFOS, BaP and BP3 following protocols described
145 in Cormier et al. [58], with minor modification for the PVC preparation. For the sorption of
146 PFOS, 200 g/L of PVC was mixed with 200 mg/L of PFOS in 1 L polypropylene bottle
147 (Lamaplast; Sesto San Giovanni, Italy), while for BaP and BP3, 125 g/L of PVC was added in
148 400 mL glass bottles (Thermo Scientific, Lund, SE) mixed with 5000 µg/L and 20 µg/L of
149 BaP and BP3, respectively. Bottles were then placed on a rotary shaker at 20 rpm for 7 days
150 (PFOS) or 2 days (BaP and BP3). After sorption, spiked MPs were filtered with a ceramic
151 funnel equipped with a 1µm Whatman R glass microfiber filter (GE Healthcare Life Sciences;
152 Uppsala, SE), then, filters were rinsed with deionized water and dried by vacuum evaporation.
153 Final concentrations of compounds on MPs are indicated in Table 1.

154 Fish were exposed to nine different treatment diets: control (Control), virgin polyethylene
155 MPs (PE-MP), virgin polyvinyl chloride MPs (PVC-MP), spiked MPs with perfluorooctane
156 sulfonate (PE-PFOS and PVC-PFOS), benzo[a]pyrene (PE-BaP and PVC-BaP) and
157 benzophenone- 3 (PE-BP3 and PVC-BP3). Diets were prepared with a final ratio of MPs of
158 1% wet weight (ww). For this purpose, MPs were mixed in a glass tube with pellets of
159 appropriate size following fish growth (see supplementary materials) using a 4 mm
160 bottlebrush fixed on a drill for 3 minutes. Afterward, to reinforce the binding of MPs to
161 pellets, flaxseed oil was added (5 µL per gram of food) and mixed for an additional 3 minutes.
162 The control condition consisted of fish fed with plain food mixed with flaxseed oil as
163 indicated above. Due to MPs size range, exposure to PVC MPs started from 2 months post
164 fertilization, while exposure to PE MPs could start at first feeding (10 dpf in medaka and 5
165 dpf in zebrafish). Fig. 1 provides a schematic overview of the experimental design of the
166 study.



167

168 Figure 1. Schematic timing of exposures to PE and PVC MPs.

169 For practical and exposure management reasons, exposures to PE and PVC MPs of marine
 170 medaka and zebrafish were performed sequentially from June 2017 to December 2018, with
 171 all sets of treatments including their respective Control replicates.

172 After four months of exposure, fish were euthanized with benzocaine (500 mg/L, Sigma) for
 173 sampling for biomarkers or chemical analyses. For this purpose, and depending on
 174 downstream analyses, entire fish or dissected tissues were immediately frozen in liquid
 175 nitrogen and stored until analysis.

176 **2.3 Chemical analyses**

177 Chemical load were analysed in fish after 1, 2, and 4 months of exposure to PE MPs and after
 178 4 months of exposure for PVC MPs. At 1 or 2 months pools of 5 fish were sampled to
 179 increase the biomass of the sample ($n=3$), later individual fish were used ($n=3-6$) to determine
 180 PFOS, BaP and BP3 concentrations in whole body. Sampling was performed at the occasion
 181 of biometries and euthanatized fish were snap frozen in liquid nitrogen, then stored in amber
 182 glass vials (for BaP and BP3 analyses) or micro tubes (for PFOS analyses) and kept at -20°C.
 183 Extraction procedures are described below while chemical analyses are described in
 184 supplementary materials.

185 **2.3.1 PFOS analysis**

186 To analyse PFOS in fish, the ion pair extraction was performed using alkaline digestion [75,
187 76]. Frozen fish were grinded in a mortar with the addition of 20 mM of NaOH, sonicated for
188 30 min and placed on a horizontal shaker overnight (16 h). The pH was neutralized with
189 hydrochloric acid, and 0.5 M of tetrabutylammonium sulphate as well as 10 ng of mass-
190 labelled $^{13}\text{C}_4$ PFOS (Wellington Laboratories Inc., Canada) were added. Samples were then
191 sonicated for 15 min. Extraction was performed three times with 2 mL methyl tert-butyl ether
192 and 15 min of sonication, followed by centrifugation 10 min at 8000 rpm. Extracts were
193 evaporated down to dryness using a N₂ stream and then, methanol was used to dissolve them.
194 Moreover, 2 ng of mass-labelled $^{13}\text{C}_8$ PFOS (Wellington Laboratories Inc., Canada) was
195 added. Limit of detection (LOD) was 10 pg/g.

196 **2.3.2 BaP analysis**

197 Frozen fish were homogenized in a mortar. Anhydrous sodium sulphate in a 1:5 ratio was
198 added to eliminate water in the sample, as well as 1 ng of BaP d₁₂. Deactivated mini-silica
199 columns were prepared with 10% of deactivated silica and anhydrous sodium sulphate,
200 washed with n-hexane:dichloromethane (3:1, v/v). Samples were eluted with 8 mL of n-
201 hexane:dichloromethane (1:1, v/v), and reduced to 200 μL before a transfer to toluene
202 (reduced to 500 μL), with the addition of 2 ng of perylene d₁₂. LOD was 5 pg/g.

203 **2.3.3 BP3 analysis**

204 Benzophenone-3 is a compound easily metabolized in organisms [77, 78], leading to the
205 formation of metabolites. Benzophenone-1 (BP1) was reported to be the major metabolite of
206 BP3, as detected in the urine of rats and humans [79, 80] as well as in zebrafish [77]. To
207 determine the concentration of BP3 and BP1, frozen fish were ground in a mortar with 1 mL
208 of acetonitrile, followed by 15 min of sonication, 15 min of horizontal shaking and 5 min on a
209 vortex. Samples were then centrifuged for 15 min at 3900 rpm and passed through a filter
210 (GHP acrodisc 13 mm). Filtrates were stored in a freezer (- 20 °C) overnight (16 h) to
211 precipitate proteins. After the precipitation, the samples were centrifuged and the supernatant
212 was evaporated down to 100 μL using a N₂ gas stream. 500 ng of BP3 d₁₀ was added as
213 standard and an external quantification was used with a calibration curve of BP3 and BP1
214 (0.04 to 80 pg/ μL). LOD was 0.05 ng/g.

215 **2.4 Molecular and cellular markers**

216 Sampled tissues (liver, muscle and brain) from both medaka and zebrafish were homogenized
217 in 0.1 M phosphate buffer (pH= 7.7; 0.1 M KCl). The homogenates were then centrifuged at
218 9000 g at 4 °C for 20 min. The supernatant (fraction S9) was then collected in a clean micro

219 tube and stored at –80 °C before being used for measurement of ethoxresorufin O-deethylase
220 activity (EROD; liver samples), thiobarbituric acid reactive substances (TBARS; muscle
221 samples), and acetylcholinesterase (AChE; brain samples). The protein concentration in the
222 S9 fraction was measured using Bradford's method with bovine serum albumin (BSA) as a
223 standard [81]. Spectrophotometric measurements were performed in a Biotek Synergy HT
224 microplate reader. To improve detection, tissue pools from 3 males or 3 females were used in
225 triplicates per sex for each treatment. To investigate DNA damage using the Comet assay,
226 blood was collected from anesthetized medaka and cryopreserved until analysis. Detailed
227 methods are given in supplementary materials.

228 **2.5 Individual markers in the F0 generation**

229 **2.5.1 Survival, growth and sampling**

230 During exposure, dead larvae or fish were monitored daily. Individual standard length (mm)
231 and body weight (mg) were measured after two and four months of exposure. For this
232 purpose, fish were shortly anesthetized with benzocaine (50 mg/L, Sigma Aldrich, France) as
233 described previously [74] or performed at sampling time (see above). Depending on time
234 point, sex and treatment, between 15 and 50 individuals were monitored per sex per treatment.
235 Adult behaviour (anxiety and activity) was monitored after reproduction assessment and
236 methods are described in supplementary materials.

237 **2.5.2 Reproduction**

238 Reproduction was monitored starting after 3 months of exposure and for approximately one
239 month. Because of biological differences, reproduction was assessed using different methods
240 in medaka (group-spawning) and zebrafish (pair-spawning).

241 Medaka groups were left undisturbed in their rearing tanks. The day before collection, tanks
242 (all replicates) were siphoned in the afternoon to remove faeces and previous eggs. The next
243 morning, within 4 hours after light onset, tanks were siphoned again and eggs were collected
244 and quickly cleaned. Afterward, they were treated and further reared if needed as described
245 above. This procedure was repeated more than ten times over one month for all replicates of
246 each treatment.

247 For zebrafish, couples were performed as described above, spawning boxes were inspected on
248 the following morning, and the presence of eggs scored as a successful attempt. In this case,
249 eggs were collected, cleaned, sorted, and transferred in Petri dishes filled with E3 as described
250 above. The general rule was to set up five pairs per condition per attempt, using fish from

251 each replicate sequentially. For exposure to PE MPs, the number of attempts ranged from 23
252 for Control to 30 for all other treatments. For exposure to PVC MPs, 53 attempts were
253 performed for Control and 60 for each other treatments.

254 When eggs were obtained, they were counted and sorted to determine the total number of
255 eggs and to calculate fertilization rates. Then 30 eggs from each spawn were further reared for
256 downstream monitoring.

257 **2.6 Individual markers in the F1 generation**

258 **2.6.1 Larval behaviour**

259 Larval behaviour was monitored at 12 dpf for medaka and 5 dpf for zebrafish (which
260 corresponds to approximately 2 days after hatching in both cases) using the larval photomotor
261 response (LPMR) test as described in [82]. Briefly and for both species, larvae were
262 transferred individually into one well of a 24-well plate (Krystal 24, opaque wall and clear
263 bottom microplate, Dutscher), and plates were transferred to an enlightened incubator at 28°C in
264 the behaviour room. Plates were then successively placed into DanioVisionTM (Noldus, NL)
265 in the dark for 10 min of acclimation before the LPMR test, which included the following 5-
266 min steps: Light on-1 (LON1, 70 lux), Light off (LOFF, <1 lux) and Light on-2 (LON2, 70
267 lux), with constant infra-red light maintained during video recording. Distance travelled (cm)
268 over the 5 min of each period was then calculated using Ethovision XT (Noldus, NL). Larvae
269 with tracking issues were removed resulting in 50 to 100 larvae analysed per treatment.

270 **2.6.2 Embryonic and larval survival**

271 For medaka, embryonic survival and hatching of collected eggs (subsample of 30 eggs per
272 treatment and dates) were monitored over 14 days. For zebrafish, subsamples of 30 eggs from
273 single spawns collected throughout the attempts were monitored over 5 days. For both
274 species, there was a daily monitoring of F1 survival and hatching and a daily exchange of
275 20% of the medium. In addition, for zebrafish, a larval survival experiment was performed,
276 without feeding, by monitoring over time the natural death of larvae. This aimed at evaluating
277 their available energy resources.

278 **2.7 Statistical analyses**

279 Normality of the data and variance homogeneity were checked using the Shapiro-Wilk and
280 Levene tests respectively. Analysis of variance was performed for normal data and followed
281 by post-hoc Fisher's or Tukey HSD tests. For data that did not fulfil normal distribution, the
282 Kruskal-Wallis non-parametric test was performed followed by multiple comparison post-hoc

283 tests to identify differences between treatments using Statistica (Tibco). For medaka, the
284 kinetic of number of eggs produced was compared between treatments by using slope
285 comparison methods (Zar (1984) in Prism software, Graphpad). Differences in F1 larval
286 survival were assessed using Log-rank (Mantel-Cox) test, and time at which 50% mortality is
287 reached was calculated using four-parameter logistic regression. Akaike's information
288 criterion was used for comparison with Control and relative virgin MP counterpart, both using
289 Prism software. All statistical analyses were carried out at a 95% level of significance and
290 values are represented as mean ± SD.

291 **3. Results**

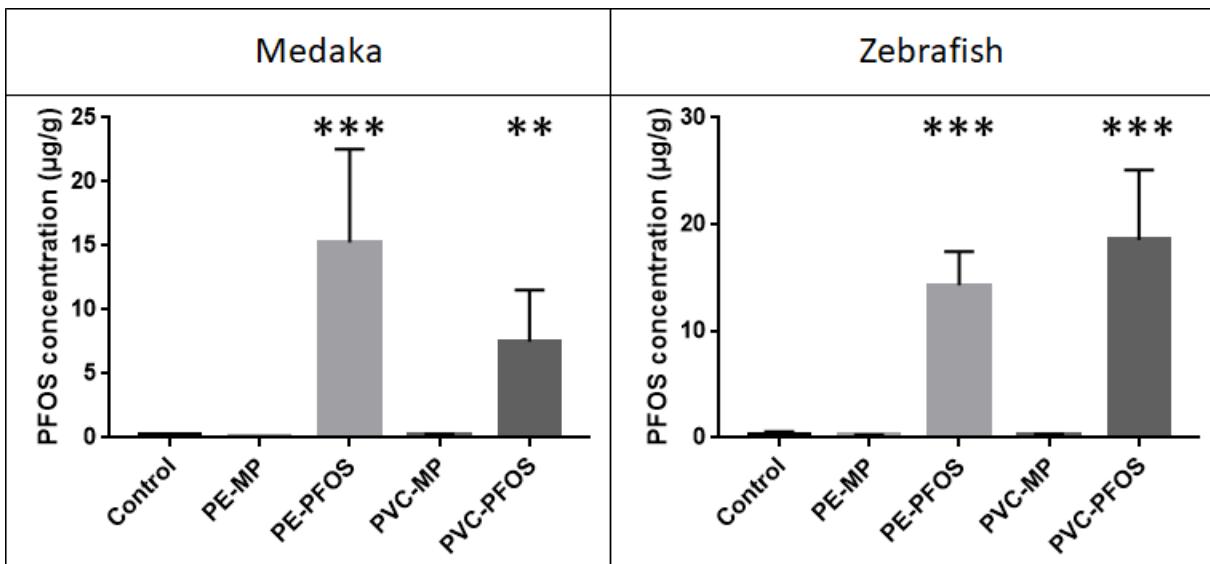
292 **3.1 Characterization of MPs and monitoring of exposure**

293 Mean particle size was 5 and 105 µm for PE and PVC MPs and their chemical compositions
294 were confirmed (Fig. S1-S2). Virgin MPs were devoid of a significant amount of the three
295 chemicals used in this study (concentration below LOD) while spiking was efficient as shown
296 in Table 1.

297 **Table 1.** Concentrations of PFOS, BaP (µg/g) and BP3 (ng/g) measured on virgin and spiked
298 PE and PVC MPs. (LOD were 10 pg/g, 5 pg/g and 5 ng/g for PFOS, BaP and BP3
299 respectively; Mean ± SD; n=3).

	PFOS	BaP	BP3
Virgin PE-MP	<LOD	<LOD	<LOD
Spiked PE MPs	70.22 ± 12.41	16.87 ± 0.22	106.00 ± 6.50
Virgin PVC-MP	<LOD	<LOD	<LOD
Spiked PVC MPs	159.54 ± 40.84	11.50 ± 1.35	107.00 ± 1.50

300
301 Concentrations of BaP and BP3 measured in fish exposed to MPs spiked with either BaP or
302 BP3 were below detection limits. BP1 was reported to be the major metabolite of BP3, as
303 detected in the urine of rats and humans [79, 80] as well as in zebrafish [77]. For fish exposed
304 to MPs spiked with BP3, BP1 was also monitored and was also below the detection limit.
305 Therefore, no sign of accumulation of these compounds was demonstrated. For PFOS, its
306 concentration in Control fish or fish exposed to PE-MP or PVC-MP was similar in the range
307 of 0.19-0.31 µg/g. PFOS concentration in fish exposed to PE-PFOS was more than 20-fold
308 higher at 7.46 ± 3.21 µg/g 8.86 ± 2.41 µg/g ww after 1 or 2 months respectively. After 4
309 months of exposure, PFOS concentration was 25 to 60-fold higher in fish exposed compared
310 to Control or virgin MPs (Fig. 2).



311

312 **Figure 2.** PFOS concentration in fish exposed for 4 months to virgin MPs (PE, PVC) or to
 313 spiked MPs (PE-PFOS, PVC-PFOS). Stars indicate significant differences from Control and
 314 their respective virgin counterpart. (Mean \pm SD; Tukey HSD; **: $p<0.01$; ***: $p<0.001$; Fish
 315 n=[3-6]).

316 3.2 Toxicity in the F0 generation

317 3.2.1 Molecular and cellular markers

318 EROD, AChE, and TBARS activities were monitored in the liver, brain, and muscle,
 319 respectively. There was no significant difference in EROD, AChE, or TBARS activity
 320 whatever the species, sex, or MPs either virgin or spiked (Table S1A-S1D). The only
 321 exception was for EROD activity measured in zebrafish males exposed to PE MPs for which
 322 there was a significant effect of treatment (Kruskal-Wallis_(4,15)=10.23, $p=0.037$) but post-hoc
 323 test revealed no difference with Control.

324 In medaka, the Comet assay performed on blood cells revealed no evidence for genotoxicity
 325 whatever sex or treatment.

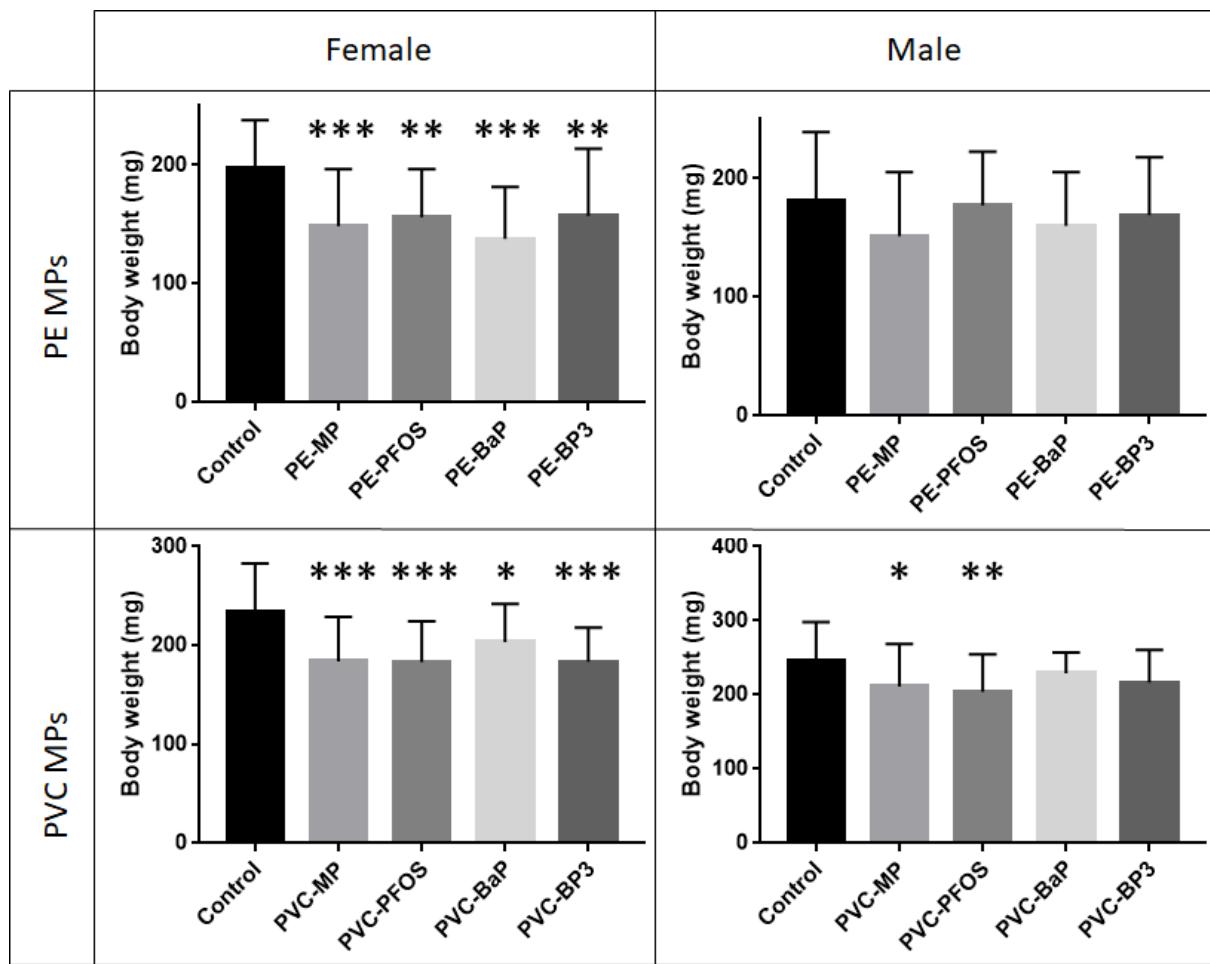
326 3.2.2 Survival and growth

327 No significant mortality was recorded whatever the treatment and no deviation from the
 328 control was observed. Biometries were performed in both fish species after approximately 2
 329 and 4 months of exposure, i.e. when PE MPs exposed fish were 2 and 4 mpf and PVC MPs
 330 exposed fish were 4 and 6 mpf (for medaka, fish exposed to PVC MPs were only measured
 331 once at 6 mpf). For the sake of clarity, the duration of exposure will be used in the text
 332 hereafter rather than age.

333 After 2 months of exposure to PE MPs, no significant difference between Control and
334 exposed fish was observed for standard length or body weight whatever the treatment (Fig.
335 S3). For PVC MPs exposures, a reduction in growth was observed in some cases. Body length
336 was not modified in males whatever the species, except for a slight decrease (-6%) in male
337 zebrafish exposed to PVC-MP. In female zebrafish, a slight decrease (less than -15%) was
338 observed for PVC-MP. Regarding body weight, a decrease (-15%) was observed in male
339 zebrafish exposed to PVC-MP and a decrease in body weight was observed in female
340 zebrafish exposed to all PVC MPs except PVC-PFOS (Fig. S4).

341 After 4 months of exposure, a significant decrease in growth was observed for males exposed
342 to some MPs and for all exposed females whatever the species and treatment (Fig. 3-4 and S5-
343 S6). In further detail, body length was not modified by PE MPs in males in medaka ($p=0.18$)
344 or zebrafish ($p=0.09$). In females, significant effects of PE MPs were observed in both species
345 ($p<0.001$ in medaka and $p=0.026$ in zebrafish). Post-hoc tests revealed a significant decrease
346 in body length of medaka exposed to PE-MP, PE-BaP, and PE-BP3 as well as for zebrafish
347 exposed to PE-BaP and PE-BP3 (-4 to -11%; Fig. S5-6). Regarding body weight (Fig. 3-4),
348 the significance of effects for the exposure to PE MPs was just above the threshold ($p=0.052$)
349 in medaka males but post-hoc tests revealed no significant differences. In zebrafish males,
350 there was a significant effect of exposure to PE MPs ($p=0.008$); post-hoc tests indicated a
351 significant decrease for PE-MP and PE-PFOS exposed males. In females, the effect was
352 greater with a decrease in body weight by 20 to 35% for all MPs, both in medaka and
353 zebrafish ($p<0.001$).

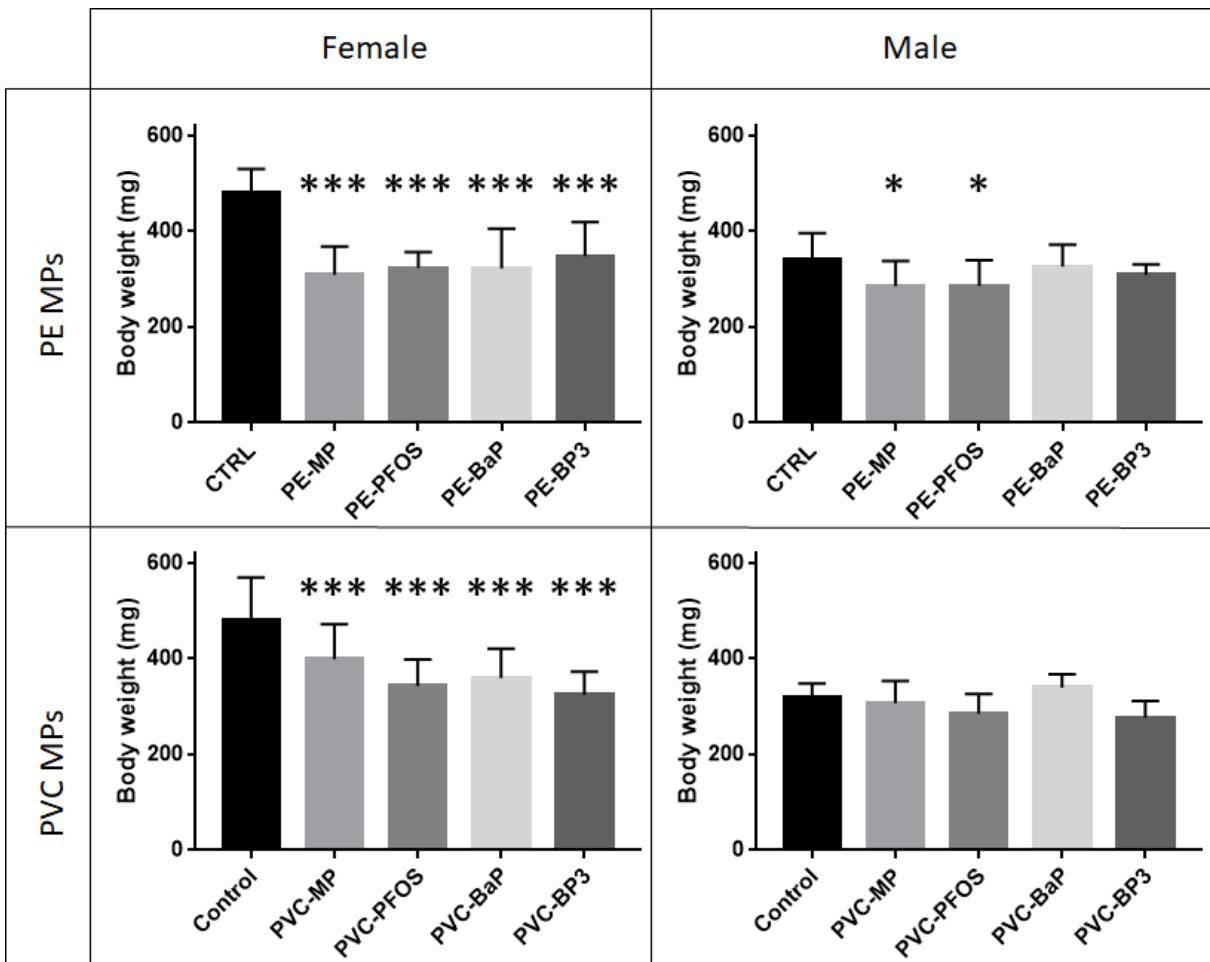
354 For PVC MPs, body length (Fig. S5-6) was altered in medaka males ($p<0.005$) with a weak
355 (<10%) but significant decrease in length for males exposed to PVC-MP and PVC-PFOS. In
356 zebrafish males, significance was just above the threshold ($p=0.057$) but post-hoc tests
357 revealed no significant differences. In females, there was a strong significant exposure effect
358 by PVC MPs in both medaka and zebrafish ($p<0.001$). Post-hoc tests revealed a significant
359 reduction in body length for all PVC MPs except PVC-BaP in medaka and all PVC-MPs in
360 zebrafish. Similar effects were observed regarding the body weight. For body weight, post-
361 hoc tests revealed a moderate but significant decrease in medaka males exposed to PVC-MP
362 (-14%) and PVC-PFOS (-16%) but no difference in zebrafish. In females, a stronger decrease
363 was observed for all PVC MPs in medaka (up to -22% for PVC-MP and PVC-PFOS) and in
364 zebrafish (up to -30% for PVC-PFOS, PVC-BaP, and PVC-BP3).



365

366 **Figure 3.** Medaka body weight after 4 months of exposure to PE and PVC, either virgin or
 367 spiked. (Mean \pm SD; Tukey HSD; *: $p<0.05$; **: $p<0.01$; ***: $p<0.001$; Fish n=[26-51] per
 368 sex per treatment from three replicates).

369



370

371 **Figure 4.** Zebrafish body weight after 4 months of exposure to PE and PVC, either virgin or
 372 spiked. (Mean \pm SD; Tukey HSD; *: $p<0.05$; **: $p<0.001$; Fish n=[25-49] per sex per
 373 treatment from three replicates).

374

375 3.2.3 Adult behaviour

376 Swimming activity and anxiety level were monitored in adults. None of the treatment (either
 377 PE or PVC, virgin or spiked) modified activity or anxiety levels of fish when compared to
 378 Control fish (Fig. S7-S8).

379

380 3.2.4 Reproduction

381 For biological reasons, the reproduction was assessed using different mating methods for
 382 medaka (group-spawning) and zebrafish (pair-spawning); hence, reproduction variables are
 383 different but spawns were obtained for both species and all treatments.

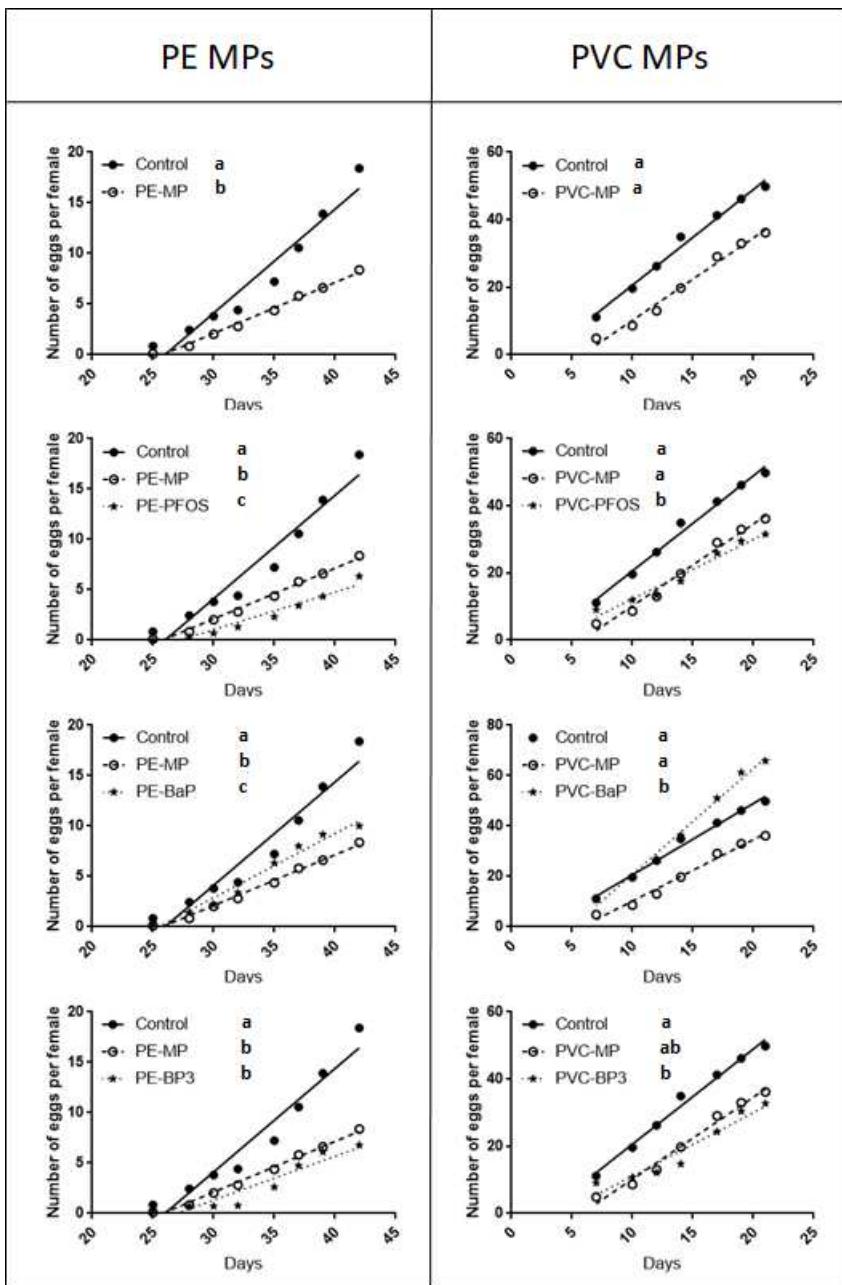
384 For medaka PE MPs exposures, we observed a significant delay in spawning onset. Fish
 385 exposed to PE-MP, PE-PFOS and PE-BP3 started to spawn 16 to 18 days later than the

386 respective Controls, while PE-BaP fish showed a 10 days delay. For PVC MPs exposed
387 medaka, such a delay was not observed but it is noteworthy that these fish (both PVC MPs
388 exposed and respective Control) were bigger than the PE MPs fish (exposed and respective
389 Control; +15-20% depending on the treatment), which may explain an earlier onset of
390 spawning and the fact that this time point was missed. A delay of 3-4 days can, however, be
391 noted when considering the number of eggs spawned (Fig 5).

392 Slopes of reproductive output over time normalised to the number of females in the tank were
393 compared (Fig 5). Slopes of reproductive output of all PE MPs were significantly reduced
394 compared to the one of Control ($p<0.01$). For Control fish of PE MPs exposure, the slope was
395 1.03 egg per day per female (95% confidence interval: [0.77-1.29]). The slope for PE-MP was
396 43% of Control ($p<0.01$). For spiked PE MPs, slopes were all below that of the Control
397 ($p<0.01$) with PE-PFOS being 31%, PE-BaP 56%, and PE-BP3 40%. When comparing with
398 the slope of reproductive output of PE-MP, an increase of 130% was measured for PE-BaP
399 ($p=0.017$) while there was a significant decrease for PE-PFOS (71%; $p=0.013$) and no
400 difference for PE-BP3 (93%; $p=0.32$).

401 For PVC MPs, there was no significant difference between Control (2.83 [2.47-3.20]) and
402 PVC-MP, albeit the slope was at 86% (2.44 [2.07-2.81]; $p=0.08$). A strong increase in the
403 reproductive output slope was observed for fish exposed to PVC-BaP (148%; $p<0.01$). For the
404 other two conditions, a significant and similar decrease in slope was observed (PVC-PFOS:
405 63%; $p<0.01$ and PVC-BP3: 67%; $p<0.01$). As for PE MPs, comparison of these slopes with
406 the one of PVC-MP confirmed the increase in slope of PVC-BaP exposed fish compared to
407 PVC-MP (172%; $p<0.0001$), and indicated a decrease of PVC-PFOS (73%; $p=0.009$) and no
408 difference for PVC-BP3 (77%; $p=0.071$).

409



410

411 **Figure 5.** Reproductive output of medaka exposed to PE and PVC, either virgin or spiked.
 412 Top panels allow comparison of output from medaka exposed to PE or PVC MPs versus their
 413 respective Control. Further panels include comparisons with spiked MPs compared to both
 414 Control and virgin MPs. The number of eggs obtained is normalised according to the number
 415 of females present in the tank. R square of linear regressions are all above 0.9. Different
 416 letters indicate different slopes between treatments.

417 Fertilisation rate was monitored for each collection of medaka eggs (Fig. S9) and there was no
 418 difference over the period of collection ranging between 71 and 80% for PE MPs (ANOVA
 419 $F_{(4, 114)} = 0.5707; p=0.684$) and ranging between 90 and 96% for PVC MPs (ANOVA $F_{(4, 108)}$
 420 $= 0.6257; p=0.645$). No change was observed in hatching rates ranging between 44 and 65%

according to treatment (RM-ANOVA; $F_{(28, 221.36)}=0.509$, $p=0.982$ for PE MPs and $F_{(24, 172.15)}=1.152$, $p=0.293$ for PVC MPs).

For zebrafish, individual spawns were monitored. As for medaka, a delay of 2-3 weeks in the onset of spawning was observed for PVC-BaP and PVC-BP3. Reproduction was also monitored by comparing the proportion of successful spawning attempts (*i.e.* when eggs were obtained, independently of spawn size or quality). The proportion of successful spawning attempts was only significantly reduced for PE-BP3 (78.6% for Control vs. 47.5% for PE-BP3; Fisher's exact test, $p=0.01$; Fig. 6). For all PVC MPs-exposed zebrafish, spawning success was significantly reduced (50.0% for Control vs. 12.1 to 24.2% for virgin and spiked PVC; Fisher's exact test, all $p<0.01$). There was no difference between virgin MPs (both PE and PVC) and their respective spiked counterparts (Fisher's exact test, $p>0.05$).

There was no difference between Control and exposed treatment concerning either fertilisation rates ($F_{(5,71)}=0.794$, $p=0.56$ for PE MPs and $F_{(5,72)}=2.169$, $p=0.07$ for PVC MPs; Fig. S9) or spawn size ($F_{(5,71)}=1.404$, $p=0.23$ for PE MPs and $F_{(5,72)}=1.804$, $p=0.12$ for PVC MPs; Fig. S10).

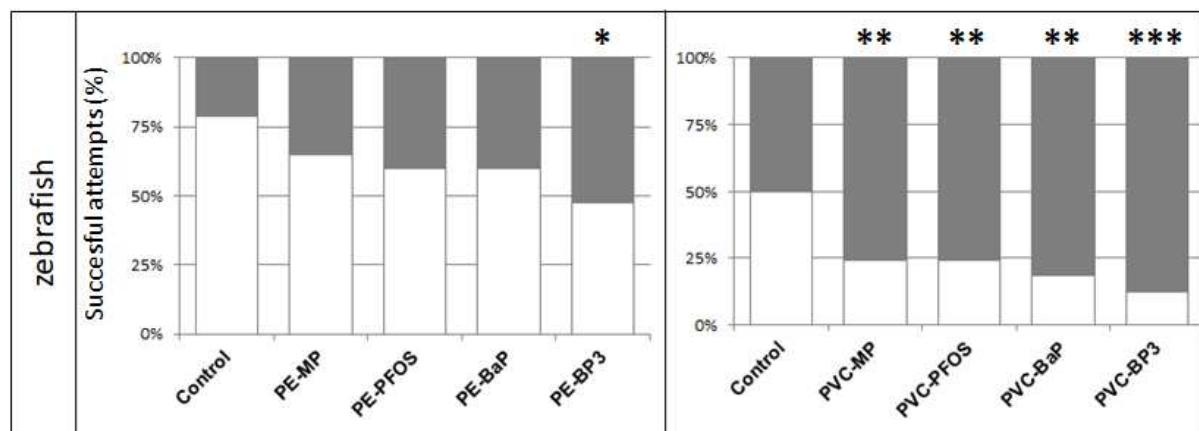


Figure 6. Relative proportions of successful (white) and failed (grey) attempts to obtain eggs from zebrafish exposed to PE and PVC MPs, either virgin or spiked. (Fisher's exact test; *: $p<0.05$; **: $p<0.01$; ***: $p<0.0001$; Attempts n=[23-60] from three replicates).

3.3 Toxicity in the F1 generation

3.3.1 Larval survival

Larval survival was monitored on unfed larvae in zebrafish only. Survival was first overall analysed, then the age at which 50% of mortality was reached was calculated. All treatments affected larval survival with the exception of PE-BaP (Chi^2 value=2.98; $p=0.08$) and PVC-BaP (Chi^2 value<0.01; $p=0.98$) (Table S2). Because the Log-rank test appeared quite permissive, we further characterized survival curves by calculating the age at which 50% of

447 mortality occurred (Fig. S11). This was 11.37 dpf [95% CI: 11.35-11.39] for Control larvae.
448 Values for PE-BaP, PVC-BaP and PE-BP3 did not differ significantly from Controls. Values
449 for PE-PFOS and PVC-MP were significantly reduced by 12.0 and 13.2 hours, respectively.
450 Values for PVC-PFOS and PVC-BP3 were significantly but marginally reduced by 4.08 and
451 3.6 hours respectively. On the other hand, survival of PE-MP larvae was significantly
452 prolonged with 50% of mortality occurring 13.2 hours later than Control larvae.

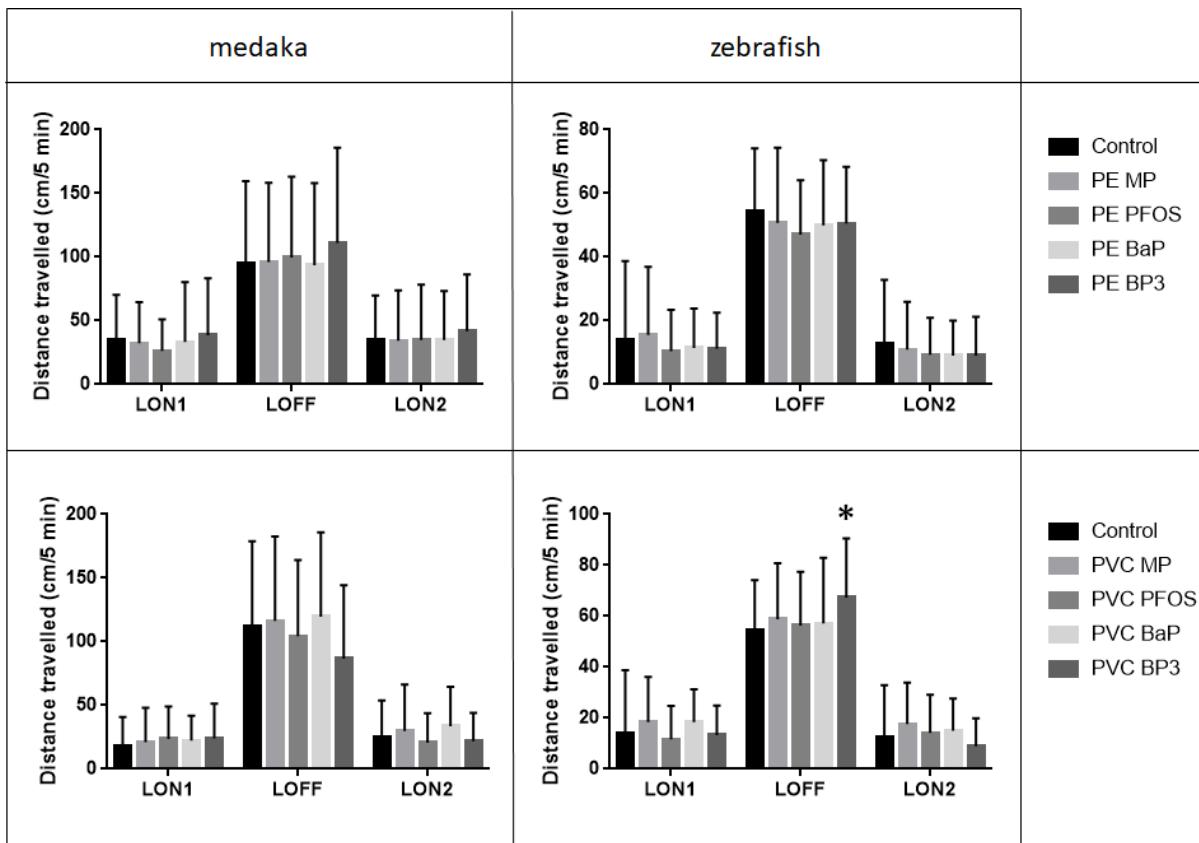
453 **3.3.2 Larval growth**

454 Larval growth was evaluated at 14 dpf in medaka and at 5 dpf in zebrafish (Fig. S12). In
455 medaka, there were some differences in standard length of offspring of fish exposed to PE
456 MPs ($F_{(4, 228)} = 7.261; p<0.0001$) and PVC MPs ($F_{(4, 158)} = 4.151; p=0.0032$), but post-hoc
457 revealed only few small differences. Indeed, PE-MP F1 larvae were slightly shorter (-4%)
458 than Control larvae, while PVC-BaP F1 larvae were slightly longer (+5%). In zebrafish F1
459 larvae, there was no significant difference between treatments (Kruskal-Wallis $_{(12, 135)}=16.47$,
460 $p=0.170$).

461 **3.3.3 Larval behaviour**

462 The behaviour of offspring was evaluated at 14 dpf in medaka and 5 dpf in zebrafish using the
463 LPMR assay. Control larvae displayed a regular activity pattern, with an increase in activity
464 during the light off (LOFF) separating the two light on periods (LON1 and LON2) (Fig. 7).

465 For medaka, there was no significant difference between PE or PVC MPs and their respective
466 controls (RM-ANOVA $F_{(12, 1492.5)}=0.968, p=0.478$, and $F_{(12, 778.14)}=1.620, p=0.081$). In
467 zebrafish exposed to PE MPs, there was no difference in activity pattern when compared to
468 Control within each period (RM ANOVA $F_{(12, 817.83)}=0.757, p=0.695$). For PVC MPs, there
469 was a significant difference between treatments (RM ANOVA $F_{(12, 934.24)}=3.136, p<0.001$) and
470 the post-hoc analyses revealed that there was an increase in activity of PVC-BP3 compared to
471 their Control during the LOFF period ($p< 0.005$).



473 **Figure 7.** LPMR in F1 larvae issued from fish exposed to PE and PVC, virgin and spiked
474 PVC. Distance travelled over 5 min periods. Data are given as means \pm SD (n = 50 to 100
475 larvae per treatment). (RM-ANOVA; *: $p < 0.05$).

476 4. Discussion

477 In general, particles that are identified in the lumen of the digestive tract of marine biota are in
478 the higher range of MPs size, smaller ones often being ignored for technical reasons, and
479 because their number is low, e.g. 1 to 5 particles per fish [4, 48]. Egestion of MPs by animals
480 in general, and fish in particular, is quite fast [34]; nevertheless, the presence of MPs has been
481 observed in a large proportion of sampled individuals of a large number of fish species
482 captured in the oceans, seas, and freshwaters [48, 83-87]. Altogether, this suggests that in the
483 wild, exposure is almost constant over time and that the number of MPs ingested by fish is
484 largely underestimated. This is particularly true for small MPs. In this experiment, we
485 exposed fish to food pellets spiked with PE or PVC MPs at a 1% ww of diet. To envision
486 ecological relevance, we related the weight of given MPs to that of a cubic particle of 330 μ m
487 on each side, which is the lower threshold of particles commonly collected with the manta
488 trawl. For an adult fed at 3% of its body weight this represented between 3.5 and 2.5 particles
489 given per day per individual, for PE and PVC respectively. Therefore, even if it is difficult to
490 compare with an environmental density of MPs, exposure conditions fell within

491 environmental ranges. These conditions were also comparable with ratios used in previous
492 experiments using a similar exposure route (0.2-2%) [56, 88-90]. It is however important to
493 mention that the actual number of the particles incorporated into the food was approximately
494 1500-fold greater for PE MPs than for PVC MPs because of the difference in particle size
495 between PE and PVC MPs. Both, the size of particles and their number may influence their
496 toxicity. Albeit quantification of MPs in digestive tract of exposed fish was not performed, it
497 has been repeatedly reported that exposure to fluorescent MPs either trophic [34, 36, 37, 56]
498 or waterborne [91, 92] resulted in actual ingestion of MPs by fish.

499 The importance of MPs as a vector for organic pollutants is still under discussion and,
500 according to modelling methods this exposure is suggested to be negligible compared to other
501 sources [39, 68]. However, as discussed by Hartmann et al. [93], it is likely that the actual
502 occurrence of MPs in the environment is underestimated due to current technical limitations
503 of the sampling methods and that MPs are not evenly distributed in the environment. In
504 addition, it seems that the ability to act as vector depends on both chemicals and MPs polymer
505 [94, 95]. Therefore it is likely that, at least in some cases, MPs may act as actual vectors for
506 organic pollutants. In addition, many chemicals are used as additives and can therefore be
507 inherently present in plastics since their production, which is the case for PFOS and BP3.
508 Besides, actual transfer of organic pollutants from MPs to biota has been extensively
509 demonstrated [34, 37, 54, 89, 96-100]. Desorption of chemicals from MPs in the digestive
510 tract and chemical transfer to tissues was reported for several chemicals. The use of
511 fluorescent MPs demonstrated that such chemical transfer was independent of MPs
512 translocation into the organism's tissues e.g. for BaP [34, 37].

513 To the best of our knowledge, no information is available for concentrations of PFOS and
514 BP3 adsorbed to MPs collected in the environment. There are some scarce reports about
515 polycyclic aromatic hydrocarbons (PAHs), with concentrations ranging from less than 0.1 to
516 more than 46 µg/g of MPs [101-106]. Concentrations of individual PAHs can reach almost 2
517 µg/g, with concentrations of BaP being up to 0.5 µg/g. The concentration of BaP used here is,
518 therefore, higher than environmental concentrations. Our results show that exposure to MPs
519 spiked with PFOS induced the bioaccumulation of the compound in fish body for both
520 polymer types. On the contrary, neither BaP nor BP3 were detected, not surprising since these
521 compounds are known to be metabolized including in zebrafish for BaP [107] and BP3, partly
522 converted to BP1 [77]. In both cases, metabolites are quickly excreted and so do not
523 accumulate.

524 The toxicity of MPs, either virgin or spiked, has been studied in various organisms, including
525 fish. In most cases, classical ecotoxicology biomarkers are used and outcomes are very
526 variable including modification of these biomarkers with particular activation of detoxication
527 and inflammatory processes, oxidative stress, and induction of cellular necrosis [47, 49, 50,
528 53 , 54]. A growing number of articles also report that exposure to MPs has no or very limited
529 effects on fish considering the same biomarkers [35, 45, 56-60, 88]. Studies reporting toxic
530 effects of MPs at the tissue level are even scarcer. Exposure to MPs was shown to produce
531 intestinal lesions in fish [90, 108, 109] while in other cases no inflammation or tissue lesions
532 were observed [35, 56, 88]. Variability in these biological responses may be due to the variety
533 of MPs and their weathering status, exposure route, exposure duration, or concentration.
534 Regarding the latter, it is to note that exposure concentrations are often very high, e.g. mg/L
535 or up to 30% of the diet portion, poorly related to environmental situations. Further, in many
536 cases, exposures are short, focused on one developmental stage, and again may not be
537 representative of life-long exposure occurring in the wild. In the study reported here, exposure
538 lasted at least 4 months and included several developmental stages, from larvae to adult for
539 PE and from juvenile to adult for PVC. As far as we know, this is the longest experimental
540 exposure of fish to MPs, but we nevertheless could not observe alterations of the biomarkers
541 monitored (EROD, TBARS, AChE) or induction of genotoxicity.

542 The present study used lifelong exposure to enable the identification of growth defects in
543 exposed fish. There are several notable elements regarding these defects: 1) 2 months
544 exposure resulted in no or much weaker effects than 4 months; 2) growth reduction was
545 similar for all MPs PE and PVC, virgin or spiked, and so was not related to extrinsic
546 chemicals; 3) growth reduction was mainly visible in females and 4) effects were similar in
547 both species. A long exposure duration was necessary to observe growth defect. This could
548 explain why no effect on growth was reported for shorter exposures to MPs in previous
549 studies [35, 56, 58, 90]. According to their smaller size (and consequently higher number
550 distributed), a higher toxicity of PE MPs would have been expected, which is not the case.
551 This may be due to additives included in PVC particles. The similar effects of PE and PVC
552 MPs suggests that neither particle size (in the studied range) nor particle numbers have
553 influenced growth disruption, or that they are compensated by polymer type-associated
554 toxicity. The similarity between virgin and spiked MPs, either PE or PVC, suggests that the
555 effect of exposure to MPs on growth is not related to chemicals burden either spiked or
556 additives and argues in favour of the hypothesis of energy budget unbalance [110]. This

557 hypothesis would fit with the major difference observed between effects in females and males
558 in both species. Indeed, in several fish species, mature females have higher food consumption
559 and/or metabolic costs compared to males as this was shown in e.g. northern pike, walleye,
560 yellow perch, catfish, or zebrafish [111-114]. Therefore, disruption of energy budget due to
561 less food consumption and/or assimilation, plus an increase in metabolic costs or dysbiosis
562 reported for numerous organisms [29, 38, 44, 45, 91, 115-118] would have larger
563 consequences in females than in males.

564 We observed no change in behaviour of directly exposed fish whatever the MPs and the
565 species. Comparisons with previous publications are not easy since contradictory effects have
566 been reported as decrease [91, 119] or increase [92], or no change in swimming activity [120].

567 Studies of the effects of MPs exposures on reproduction are scarce. Such effects on
568 reproduction were already reported in Pacific and Pearl oysters after more than 2 months of
569 exposure [118, 121]. In fish, there are only a few reports pointing towards disruption of
570 reproduction [90, 122, 123]. As for growth, there are several notable elements regarding
571 reproduction defects: 1) reproduction output was reduced in most cases upon exposure to MPs
572 but no change was observed in spawn quality; 2) there were differences between polymers
573 and virgin or spiked MPs and 3) there were differences between marine medaka and
574 zebrafish. Regarding reproductive output, in medaka there was a decrease in the number of
575 eggs produced per female per day upon exposure to almost all MPs. This situation contrasts
576 with zebrafish for which a decrease in reproduction success was observed for PE-BP3 only,
577 and for all PVC MPs. When compared to previous reports, these results differ with equal
578 number of eggs spawned after a 21-day long waterborne exposure of Japanese medaka adults
579 to polyester or polypropylene microfibers [123], but are in agreement with the decrease in
580 spawned eggs number after waterborne exposure of marine medaka or Japanese medaka to 10
581 µm fluorescent polystyrene [90, 122]. It is to note, however, that in both cases, this decrease
582 corresponds to only one collection time point after 2 or 2.5 months of exposure. Spawn
583 quality reported in these articles was mostly unchanged, as we also observed.

584 In medaka, a delay in spawning onset was observed for almost all MPs treatments while in
585 zebrafish this was only the case for PVC-BaP and PVC-BP3. Along with this delay in
586 spawning onset, co-occurs the decrease in reproduction output which was almost general in
587 medaka while occurring mainly with PVC MPs in zebrafish. These results point out
588 sensitivity differences between species responses, which appeared to be stronger in marine
589 medaka compared to zebrafish. This is not supported by the previous comparison of

590 sensitivity between both species for reproduction traits, so it is not possible to say that this is
591 related to a higher sensitivity of marine medaka to reprotoxic compounds in general, or to
592 MPs in particular. Beyond species differences, it should be noted that another major
593 difference is the salinity of the environment. Comparison of sensitivity according to salinity
594 was made using species sensitivity distributions and differences vary according to chemical
595 groups [124, 125]. In general, organic chemicals appear more toxic to saltwater species,
596 however, the underlying reasons are not clear.

597 Considering only zebrafish leads to the conclusion that PVC MPs are more reprotoxic than PE
598 MPs and that PVC is the major driver for toxicity, while for PE, BP3 is responsible for
599 reprotoxicity. The same hypothesis is not true when considering medaka for which exposures
600 to PE MPs had more severe effects than PVC MPs, effects of both plastic types being partially
601 modified by spiked chemicals. In both cases, these modifications can be an increase (BaP) or
602 a decrease (BP3 or PFOS) in reproduction output. Dietary exposure to BaP, albeit at much
603 higher concentration, resulted in no modification of reproduction output [126, 127] which is
604 in agreement with the absence of specific reprotoxicity of BaP after exposure to either PE-
605 BaP or PVC-BaP. It is noteworthy that chronic waterborne exposure of fish to PFOS with
606 PFOS body burden similar to the one obtained in the present work was reported to result in
607 physiological disruption indicative of endocrine disruption [128, 129]. This may provide a
608 mechanism underlying effects of PFOS spiked MPs on reproduction, even if the lowest
609 concentration used in these studies (0.6 µg/L) is at the level of the highest monitored
610 concentrations for PFOS. Endocrine disruption was also observed in fish waterborne-exposed
611 to BP3 but at concentrations three- to tenfold higher than the highest environmental
612 concentrations [78, 130].

613 PE and PVC MPs (both virgin and spiked) were equally able to decrease growth after
614 approximately 4 months of exposure, while it appeared that reprotoxicity of PVC was higher.
615 This difference in response between polymers types and depending on endpoint has recently
616 been shown in *Daphnia sp.* [65]. In this latter study, polyurethane and polylactic acid MPs
617 (native and from which chemicals were extracted using methanol) are toxic for some
618 measured endpoints while the extracted chemicals are not, contrary to PVC for which
619 extracted chemicals are toxic but not the MPs (native or extracted), and all MPs being more
620 toxic than kaolin particles [65]. Since there were differences between PE and PVC MPs (both
621 virgin and spiked) it is difficult to definitely assign reprotoxicity to either MPs or spiked

622 chemicals, and the interference between both. Dedicated experiments are needed for this
623 purpose.

624 In order to fully describe the consequences of a long-term exposure on population
625 recruitment, some offspring traits were monitored. Some disruptions were observed in F1
626 from exposed fish but they were not major. The slight reduction in time needed to reach 50%
627 mortality may be related to a decrease in the energy initially stored/provided to eggs. This was
628 however not translated in early developmental defects since larval growth and behaviour were
629 mostly not altered. Regarding this latter trait, the increase in response to LOFF period
630 observed in F1 from PVC-BP3 exposed zebrafish is quite important with an increase of 24%
631 compared to control larvae which may be detrimental for their survival. Disruption in
632 behaviour during LOFF has also been described as an indicator of increased anxiety and/or
633 neurotoxicity [131]. It is noteworthy that no such difference was observed in marine medaka
634 but because of a later hatch in medaka, larval behaviour assessment is done much later (14
635 dpf) compared to zebrafish (5 dpf). We can therefore make the hypothesis that this effect,
636 observed only in PVC-BP3, is related to maternal transfer of BP3, which may be eliminated in
637 medaka larvae. This would be in agreement with a previous report which has shown that BP3
638 disrupt marine medaka larval behaviour [59].

639 In other experiments, we have used the same PE MPs, virgin or spiked, to evaluate acute
640 toxicity using regulatory tests in zebrafish and medaka embryos [57-59]. The conclusion is
641 that no acute toxicity was observed at environmentally relevant concentrations. This indicates
642 that the toxicity of MPs comes from chronic exposure and so that long exposures are
643 necessary to properly assess MPs toxicity which cannot be evaluated using regulatory tests
644 using acute toxicity endpoints.

645 In conclusion, our study presents clear evidence that a reduction in growth for females and a
646 decrease in reproduction output co-occur upon chronic exposure to MPs, and that combination
647 may have important negative consequences on individual abilities to contribute to
648 recruitment, which may lead to adverse effects at a population level. Our results fit well with
649 the tentative adverse outcome pathway proposed recently to link exposure to MPs to
650 population consequences involving disruption of energy budget [110] and further work is
651 needed to evaluate this in detail. It is important to note that these effects depend on polymers
652 type (PVC being more toxic than PE), the presence or not of chemicals (BP3 being more toxic
653 than PFOS or BaP) and the duration of exposure. Regarding this last point, our result clearly
654 advocate long-term, chronic exposures as reliable for MPs toxicity evaluation in fish. In

655 addition, further studies are necessary to better understand the influence of size and polymer
656 type on MPs toxicity, as well as the role of additives. Globally, we observed no major
657 difference between both studied species but more similar comparative studies like this one are
658 necessary to conclude on the genericity of our findings.

659 **Conflict of Interest**

660 The authors declare that the research was conducted in the absence of any commercial or
661 financial relationships that could be construed as a potential conflict of interest.

662

663

664 **CRediT authorship contribution statement**

665 **Bettie Cormier:** Data curation; Formal analysis; Investigation; Roles/Writing - original draft.
666 **Florane Le Bihanic:** Data curation; Formal analysis; Investigation; Roles/Writing - original
667 draft. **Jean-Claude Crebassa:** Investigation. **Mathieu Cabar:** Data curation; Investigation.
668 **Mélanie Blanc:** Investigation. **Maria Larsson:** Investigation. **Florian Dubocq:**
669 Investigation. **Leo Yeung:** Investigation. **Christelle Clérandeau:** Data curation;
670 Investigation. **Steffen H Keiter:** Conceptualization; Funding acquisition; Supervision;
671 Writing - review & editing. **Jérôme Cachot:** Conceptualization; Funding acquisition; Project
672 administration; Supervision; Writing - review & editing. **Marie-Laure Bégout:**
673 Conceptualization; Data curation; Funding acquisition; Investigation; Project administration;
674 Supervision; Validation; Roles/Writing - original draft; Writing - review & editing. **Xavier
675 Cousin:** Conceptualization; Data curation; Formal analysis; Funding acquisition;
676 Investigation; Supervision; Validation; Visualization; Roles/Writing - original draft; Writing -
677 review & editing.

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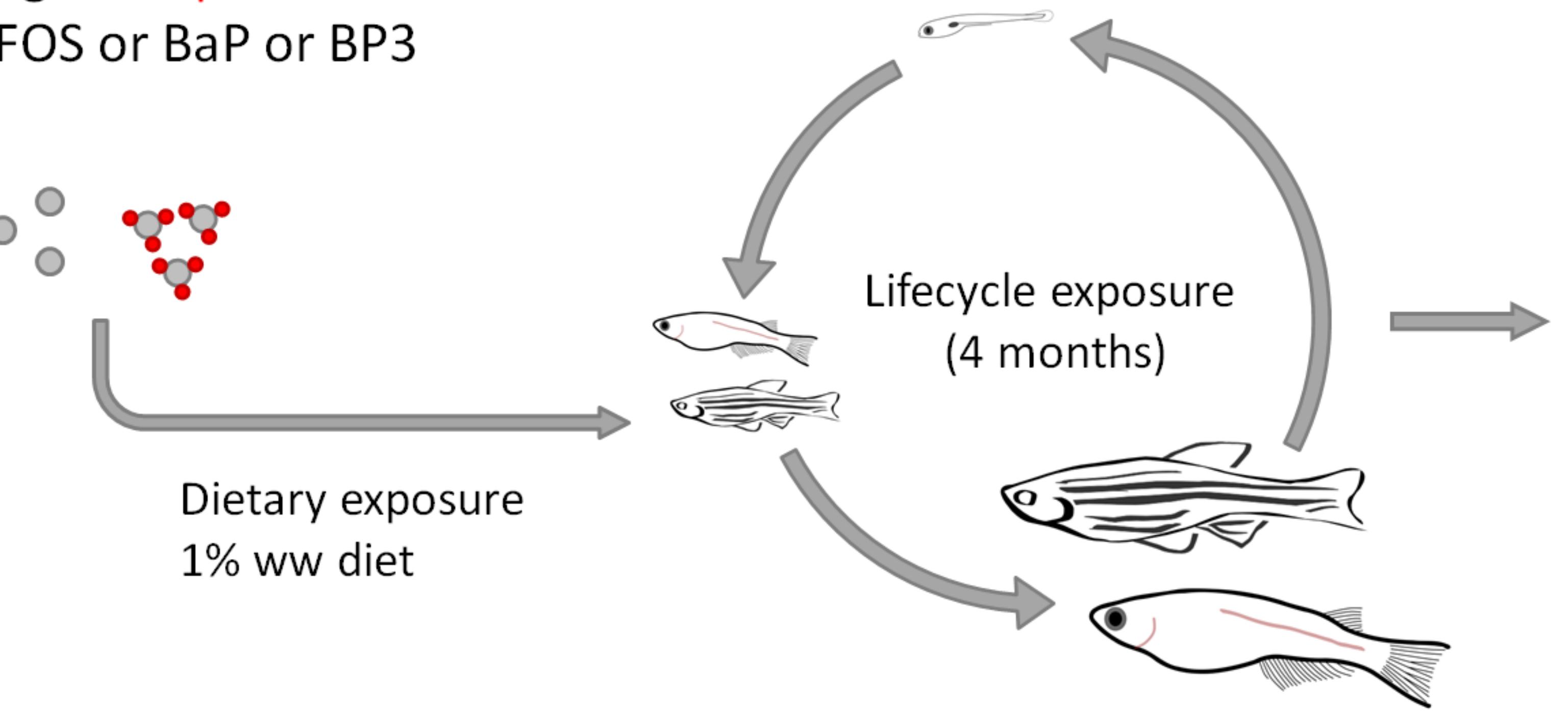
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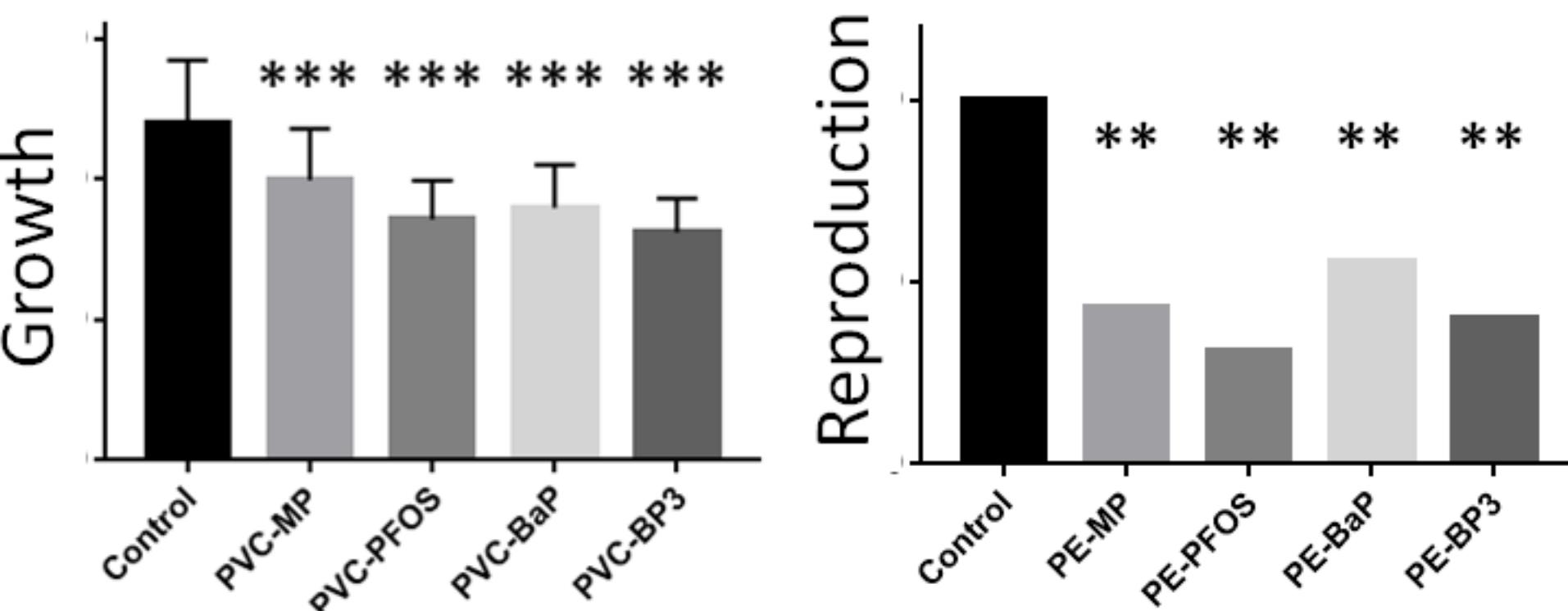
1083

Microplastics = PE or PVC
virgin or **spiked** with
PFOS or BaP or BP3



Fish= zebrafish and marine medaka

Biological disruptions



Severity varies with

- Plastic types
- Presence or absence of chemicals
- Nature of chemicals